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Special Issue Article

Stress priming affects fungal competition - evidence from a combined experimental and modelling study

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Summary

Priming, an inducible stress defence strategy that prepares an organism for an impending stress event, is common in microbes and has been studied mostly in isolated organisms or populations. How the benefits of priming change in the microbial community context and, vice versa, whether priming influences competition between organisms, remain largely unknown. In this study, we grew different isolates of soil fungi that experienced heat stress in isolation and pairwise competition experiments and assessed colony extension rate as a measure of fitness under priming and non-priming conditions. Based on this data, we developed a cellular automaton model simulating the growth of the ascomycete *Chaetomium angustispirale* competing against other fungi and systematically varied fungal response traits to explain similarities and differences observed in the experimental data. We showed that competition changes the priming benefit compared with isolated growth and that it can even be reversed depending on the competitor's traits such as growth rate, primeability and stress susceptibility. With this study, we transfer insights on priming from studies in isolation to competition between species. This is an important step towards understanding the role of inducible defences in microbial community assembly and composition.

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Introduction

Priming is a stress defence mechanism that enables an organism to remember an environmental cue and to build up an enhanced stress response to a potentially stronger future stress. Primed defence mechanisms have been observed across many microbial taxa (see meta-analysis by Andrade-Linares, Lehmann and Rillig 2016a), most of which have focused on the molecular processes that underlie priming. Complementary to research on priming processes, understanding the role of priming in stress ecology is an important step to comprehend how priming might change the effect of stressors on species fitness and community development. At the ecological level, it is still unclear how the ability of an individual to be primed, termed primeability, might influence competitive interactions and thus the community development and, vice versa, how the community context affects the benefits of priming.

Microbial priming is a defence strategy found in bacteria (Koutsoumanis and Sofos, 2004; Mitchell *et al*., 2009; Cebrián *et al*., 2010; Hernández *et al*., 2012), archaea (Trent, 1996), as well as fungi (Alvarez-Peral *et al*., 2002; Berry and Gasch, 2008; Rangel *et al*., 2008; Mitchell *et al*., 2009; Guhr *et al*., 2017). Especially fungi are suitable model organisms to study the effects of priming under different conditions, as many isolates exhibit varying degrees of primeability (Szymczak *et al*., 2020) and memory length (Diana R. Andrade-Linares *et al*., 2016b). In nature, isolated growth of fungi is rare, usually occurring only when new territory is colonized (Boddy, 2000), and fungi normally live in highly complex communities of different species that compete for space and display a broad range of mostly antagonistic interactions (Boddy, 2000; Toljander *et al*., 2006; Hiscox and Boddy, 2017), which influence community composition (Boddy, 2000, 2001). Several studies have shown that fungal combative ability is not only dependent on the species that interact but also on environmental factors such as resource availability (Stahl and Christensen, 1992; Falconer *et al*., 2008) or temperature (Boddy *et al*., 1985; Schoeman *et al*., 1996; Toljander *et al*., 2006; Hiscox, Clarkson, *et al*., 2016a), and temperature changes can

even lead to reversed competitive outcomes (Crowther *et al*., 2012). Therefore, we expect that heat priming, which affects species of distinct heat tolerance, but also distinct primeability differently in their response to heat stress, has an impact on fungal community development.

Experimental research on priming usually requires time-intensive multifactorial setups, in which organisms experience, apart from control conditions, a stress with and without preceding priming cue, as well as a priming cue without subsequent stress (Hilker *et al*., 2016). Here, simulation models can complement laboratory experiments by testing different environmental factors and species traits, e.g. imitating conditions or species combinations that could not be investigated empirically. A modelling approach thus allows a systematic investigation of distinct costs and benefits of priming for an organism. Using a mathematical model of microbes in competition, Rillig *et al*. (2015) could show that priming is beneficial more often under community conditions compared with species investigated under isolation. A follow-up study (Wesener and Tietjen, 2019) additionally showed that different strategies to reach an enhanced stress response are of different benefit. Especially the stress duration determined if an early or fast build-up of the response was most beneficial or a stronger response. However, a general understanding of how the benefit of priming can change under competition and thus influences community structure is still missing. To fill this gap, we carried out an experiment to collect dedicated data and developed a cellular automaton model simulating the growth of fungal colonies in isolation and in pairwise interactions. Our model is based on experimental data of the ascomycete *Chaetomium angustispirale* as focal species and various competitors of *C*. *angustispirale*, experiencing a mild temperature stimulus and/or heat stress. It can successfully reproduce the growth dynamics of two competing soil fungi under primed or non-primed conditions. To gather a general understanding of priming impacts on fungal competition, we systematically varied several traits of the species competing with *C*. *angustispirale* and observed how the priming benefit of *C*. *angustispirale* depends on the traits of the competitor. The specific aims of the study are (i) to identify fungal traits that affect the pay-off of priming by comparing the benefit of *C*. *angustispirale* in dual cultures with various competitors and (ii) to assess the influence of priming on competitive success.

Methods

We carried out a laboratory experiment on six soil fungal species that have been taken from the top 10 cm soil of a grassland site in Brandenburg, Germany and are thus stemming from the same soil communities. The species were grown in isolation and in dual-species mixtures to determine how the growth of species is altered by heat stress and by priming towards this stress (Fig. 1, Panels A and B). We used parts of the data to parameterize and validate the growth rates of the model (Figure 1, Panels C and D). Finally, we used the validated model to systematically assess the effect of fungal species traits and of competition on the benefits of priming as well as the influence of priming on competition between species (Fig. 1, Panel E).

Laboratory experiment of priming effects

Experimental setup. First, six soil fungal species were grown in isolation, and then *C*. *angustispirale* was grown

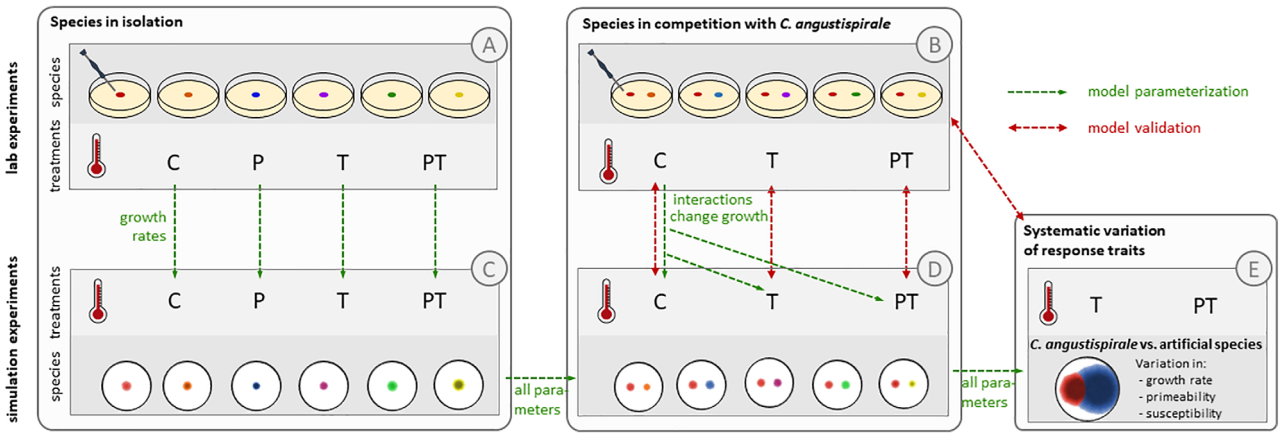

Fig. 1. Schematic overview of the experimental setup. Boxes show the performed experiments with empirical work in the upper row and simulation experiments in the lower row. Links between Parts A–E are indicated by arrows. Information on each part is given in the main text of the Methods description: (A) sections *Experimental setup* and *Determination of colony extension rates*; (B) section *Competition experiments* (C) section *Simulation model*; (D) section *Implementation of competition*; and (E) section *Simulation experiments*. Abbreviations C (control), P (priming), T (triggering), PT (priming and triggering) refer to our treatment scheme.

in competition with the other five species. All single and dual cultures were grown in a Petri dish of 90 mm diameter on potato dextrose agar in a full-factorial design of the following treatments with six replicates per fungus and treatment: (i) A control treatment (C) at constant conditions of 22°C with no disturbance, (ii) a priming-only treatment (P), in which a fungus experienced a priming stimulus of 35°C after 1 day of undisturbed growth, (iii) a triggering-only treatment (T), in which a triggering heat stress of 45°C was applied and (iv) a primed stress treatment (PT), in which the priming stimulus was immediately followed by the triggering stress. After the priming and/or triggering stimulus, the temperature was set back to 22°C. For details on the fungal species and the choice of treatment temperatures, see the Supporting Information.

The colony area was approximated by the measured diameter once per day to determine species-specific colony extension rates. Measurements were taken until the colonies reached the edges of the Petri dish or, for slower growing individuals, for 14 consecutive days.

Competition experiments. To investigate fungal growth under competition, we chose *C*. *angustispirale* as focal species competing against each of the five other species, as it showed moderate priming effects and could therefore be investigated under competition with species showing a higher and with species showing a lower primeability. That is, we use the term 'pairwise' to refer to pairs that include *C*. *angustispirale* and did not investigate competition between the other species. Plugs of mycelium of *C*. *angustispirale* were inoculated pairwise with each of the five other species equally distant from each other and from the border of the Petri dish. As soon as the two colonies touched, the same treatments on priming and triggering as in the single species experiments were applied. In pairwise cultures, the individual colony shapes were not circular and colony area was determined using ImageJ (Schneider *et al*., 2012). Scanning took place four times in total, until the Petri dish was filled. Eight replicates per competition setup and treatment were measured.

Determination of colony extension rates. We determined the species-specific growth rates by measuring the change of colony area over time for species in isolation in all four experimental treatments. Table 1 shows a quantification of the effect of the different treatments on growth (further illustrated in Fig. S1).

For the C and P treatment, a linear fit was applied to the daily diameter values of the single species experiments resulting in colony growth measured as colony diameter change [mm day^{-1}]. For the T and PT treatments, we detected the occurrence of a stress-induced lag phase, i.e. a period of no growth, and determined the duration of the lag phase and the growth of the following phase, again with a linear fit. Further details are given in the Supporting Information.

Of the six species that were primed experimentally, four did not show priming costs concerning the growth rate (Table 1, P/C), while two showed a slight overall increase in growth. To reduce the complexity of our model, we thus chose to exclude priming costs for further analyses. All fungi exhibited a lag phase without any growth under the T treatment, and four of the six species did not show a change in growth rate after the lag had ended compared with unstressed growth (Table 1, T/C). When being primed, the lag phase was shorter in five species and remained equal in *M*. *elongata*, and the growth rate after the end of the lag phase did not differ (Table 1, PT/T). Again, to avoid unnecessary model complexity, we assumed no difference in the growth rate after stress-induced lag phases for both T and PT treatments, as the changes were only marginal.

Simulation model

To simulate a fungal colony growing in a Petri dish, we developed a cellular automaton model and introduced the experimentally determined growth rates into this model. In the following section, we describe the model and how we converted the measured growth rates of the experiment, which are continuous over time and space, to the necessary discrete units of time and space of the cellular automaton.

Our model represents a Petri dish, i.e. a circular area, with an inner diameter d = 86.5 mm containing one or two fungal colonies. The area of the Petri dish is divided into square grid cells with a side length of r_{spat} = 0.5 mm, leading to 173 grid cells along the diameter of the Petri dish. To mimic the laboratory experiments, the initial colony diameter of a fungus is set to d = 6 mm. Colonies in isolation are placed into the centre of the Petri dish. Colonies in pairwise experiments are placed on the horizontal diameter equidistant from each other and the border of the Petri dish.

Simulation of cell division and colony growth follows the cellular automaton model of Gerlee and Anderson (2007). To realize radial extension of the initial colonies, each grid cell of the model is assigned one of two states: empty or occupied by a fungal cell. Fungal cells conduct cell division, i.e. produced new daughter cells in an empty neighbouring grid cell, leading to an increase in colony area.

The temporal resolution r_{temp} is 1 h. To match measured growth rates, it is necessary to determine the frequency of cell division, for which we introduce a linear increasing maturation value $m(t)$ for each fungal cell. Cell division occurs when a cell reaches a maturation age of

Table 1. Experimentally measured values of growth rates and their relative changes and lag phases after a stress stimulus.

Species (competitor number)	Control growth rate g_{si} [mm day^{-1}]	Rel. change in growth T/C	Rel. change in growth PT/T	Rel. change in growth P/C	Non-primed lag phase $L_{si,T}$ [day]	Primed lag phase $L_{si,PT}$ [day]
C. angustispirale	9.38	1	1	1	3.17	1.9
F. sp. *(1)*	6.63	1	1	1	0.71	0.01
Amphisphaeriaceae strain *(2)*	7.13	1.15 (**)	1	1.10 (*)	1.577	1.03
P. sapidus (3)	3.58	1	1	1	1.93	1.56
F. oxysporum (4)	10.57	0.91 (**)	1	1.046 (*)	0.55	0.12
M. elongata (5)	14.74	1	1	1	2.82	2.82

The significance levels between growth rates were assessed by a paired *t*-test $^*P \leq 0.05$, $^{**}P \leq 0.01$, relative changes of 1 indicated a non-significant change in growth rate. The comparison between C and P treatment showed priming costs inflicted on a primed species, comparing C and T treatment revealed the effect of stress on growth and comparing PT and T treatment quantified how much better a species grows if primed before stressed.
Abbreviations: C, control treatment without stress; P, primed treatment with a mild stress, T; triggered treatment with a strong stress; PT, primed and triggered treatment.

$m \geq 1$. The increase in m, Δm, is calculated based on the measured growth rate g_{si} relative to the temporal and spatial resolution:

$$\Delta m = \begin{cases} 0, & t_{\text{treatment}} < t < t + L_{si,\text{treatment}} \\ \dfrac{g_{si} \cdot r_{\text{temp}}}{2 \cdot r_{\text{spat}}}, & \text{else} \end{cases} \quad (1)$$

with *si* referring to the simulated species and "treatment" referring to the triggered only or primed and triggered treatment. The growth rate is corrected by factor ½, since the measured data of g_{si} include diameter growth into two directions, while simulated cell division for each side of the colony periphery is calculated separately. In case of heat stress (T or PT treatment), the maturation value remains constant for the duration of the post-stress lag phase, which starts after the application of a heat pulse (at t_T) or a priming stimulus followed by a heat pulse (at t_{PT}), and ends after the duration of the lag phase ($L_{si,T}$ or $L_{si,PT}$ respectively).

When a fungal cell reaches maturation age of $m \geq 1$, the cell divides and fills a random empty neighbouring grid cell (Moore neighbourhood with the eight surrounding cells) with a higher priority on the immediate four neighbouring grid cells, leading to an increase in area. The maturation value is then reduced by 1. The average division number corresponds to the measured radial colony extension. The daughter cell inherits its mother's new maturation age adjusted by a random variation term with a standard deviation of $\sigma = m/2$. If at the point of division none of the neighbouring grid cells is empty, the division failed and the maturation value of that fungal cell no longer increases.

Apart from competition for space, no other forms of interactions are included. The model setup leads to deadlock as only possible competitive outcome, which is a clear separation of space between competing species and the most common competitive outcome of mycelial interactions (Stahl and Christensen, 1992; Schoeman *et al*., 1996; Hiscox *et al*., 2018).

The cellular automaton model was implemented in NetLogo 6.1.0 (Wilensky, 1999) and analysed using R (R., 2018) and the nlrx package (Salecker *et al*., 2019).

Implementation of competition. Because fungi change their growth rates in dual cultures depending on their competitor (Stahl and Christensen 1992), we adjusted the growth rate $g_{si,ci}$ of species *si* under competition with competitor *ci*. For this, we applied a fit to non-stressed conditions (C), and only on data points before both fungal colonies touched to explicitly refer only to the effects that arise due to interactions at distance, as competition for space is already implemented in the model. The so-determined effect of competition was without further adjustment applied to the stress treatment (T) and primed-and-stressed treatment (PT) (shown as the green line in the right panel of Fig. 1). To validate the model, we simulated the growth of *C. angustispirale* as focal species in isolation and under competition with each one of the other five fungi under C, T and PT treatments.

Simulation experiments

As model output, we determined the colony area $A_{si} = N_{\text{cells}} \cdot r_{\text{spat}}{}^2$ [mm^2], which serves as a measure of fitness. The relative benefit of priming b_{si} for *C. angustispirale* was then described as the colony area of a fungus under stress (T) compared with the area of a primed colony under stress (PT):

$$b_{si} = \frac{A_{si,\text{PT}}}{A_{si,\text{T}}} \quad (2)$$

A value of $b_{si} > 1$ thus refers to a situation where a species performs better with priming than without.

To assess model performance, the colony area of *C. angustispirale* growing in competition with a competitor, as well as the simulated relative benefit from priming was compared with experimental data.

Subsequently, the sensitivity of this relative benefit of priming towards the following three response traits (summarized in Table 2) was evaluated in a full factorial experiment: (i) The intrinsic colony extension rate in isolation g_{si} [mm day^{-1}], (ii) the stress susceptibility defined as the length of the fungistatic lag phase under heat stress $\text{sus}_{si} = L_{si,\text{T}}$ and (iii) the primeability of a species describing the reduction of the lag phase if primed before stressed $\text{prim}_{si} = 1 - \frac{L_{si,\text{PT}}}{L_{si,\text{T}}}$. Both growth rate and stress susceptibility are absolute measures, while the primeability of a species is a relative value. By varying the response traits of the competing species, we could cover a broad range of possible competition scenarios that go far beyond the capacity of laboratory experiments.

To set a baseline, we first simulated the growth of *C. angustispirale* in competition with an identical species until the Petri dish was filled or up to a maximum of 15 days. We then systematically varied the three response traits of the competitor under T and PT conditions. Because the growth of *C. angustispirale* under competition proved to be variable depending on its competitor, we also varied the growth rate of *C. angustispirale*, while all other trait values of *C. angustispirale* remained fixed.

Second, to determine which competitor benefits more from priming, we measured the competitive shift $c_{C.a}$ of *C. angustispirale*

$$c_{C.a} = \ln\left(b_{C.a}/b_{\text{competitor}}\right) \quad (3)$$

which compares the benefits of both competitors and describes the influence of priming on competition, i.e. if the ratio of colony sizes of the two competing species is altered by a priming stimulus. Both the relative benefit of priming b_{si} and the competitive shift c_{si} depend on the performance of both competing species. However, while the relative benefit describes the potentially improved performance of a primed versus a non-primed species under competition, the competitive shift describes which of the two competitors benefits more from priming. A value of $c_{c.a} = 0$ refers to no change in colony ratios between T and PT treatment, i.e. both competitors benefit equally from priming in this competitive situation. For a value of $c_{C.a} > 0$, the colony size of *C. angustispirale* increases more than the one of its competitor.

Measuring both the relative benefit of *C. angustispirale* $b_{C.a}$ and the competitive shift $c_{C.a}$ allowed us to investigate whether certain parameter combinations affected these values differently, e.g. led to high priming benefit of *C. angustispirale* but still decreased its competitive strength because the competitor benefitted even more. We also compared the competitive shift that the model predicts for the five pairs with the experimental data to further validate our simulation model.

Results

After model fitting, we first validated the model by comparing the simulated output with experimental data of competition treatments not used for model parameterization. We then systematically varied different traits of an artificial species competing with *C. angustispirale* and assessed the benefit that *C. angustispirale* gained from priming. Additionally, we measured the effect of priming on competition strength under stress conditions.

Model validation

With our model, we could well predict the growth in competition of four of five fungal pairs under stress with and without preceding priming cue (see Fig. 2 and Fig. S3; see Fig. S4 for an observation vs. prediction plot). In

Table 2. Model parameters and their description.

Parameter	Description	Source	Unit
g_{si}	Intrinsic colony extension rate in isolation	Measured	mm day^{-1}
$g_{si,ci}$	Intrinsic colony extension rate under competition	Measured	mm day^{-1}
$L_{si,\text{T}}$	Duration of a phase of no growth after a 2 h heat stress	Measured	day
$L_{si,\text{PT}}$	Duration of a lag phase of no growth if primed before stressed	Measured	day
sus_{si}	Stress susceptibility	$\text{sus}_{si} = L_{si,\text{T}}$	day
prim_{si}	Stress primeability (reduction of the stress-induced lag phase if primed)	$\text{prim}_{si} = 1 - \frac{L_{si,\text{PT}}}{L_{si,\text{T}}}$	–

A primeability value of $\text{prim}_{si} = 1$ describes full primeability, i.e. a reduction of the lag phase to zero, while a primeability of $\text{prim}_{si} = 0$ applies to non-primeable species that exhibit the same lag phase under T and PT treatment.

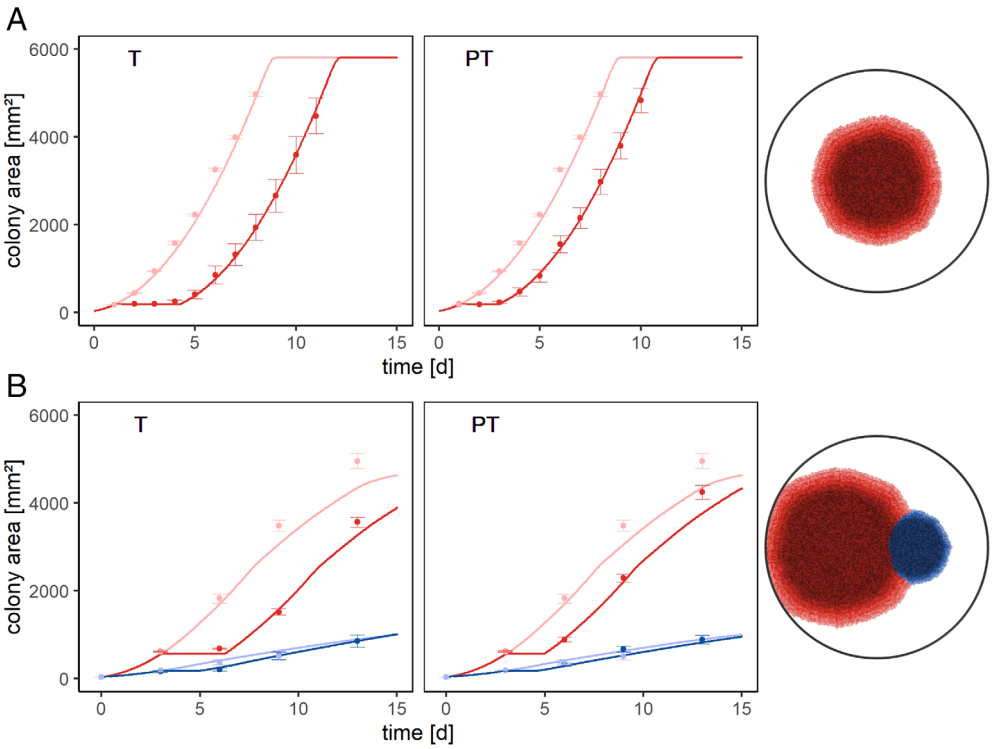

Fig. 2. Measured and simulated growth dynamics of *C. angustispirale* (red) in (A) isolation and (B) competition with *P. sapidus* (blue). Points describe empirical measurements, and lines are the corresponding simulation model output. Light shades represent the control treatment, while darker shades represent the respective stress treatments (stressed, T, or primed and stressed, PT). Error bars show the standard error of the mean of the observed data. Examples at the right show the corresponding output of the cellular automaton model on day 8.

these successful cases, the effects of interactions and stress were additive. When competing with *M. elongata*, however, the model underestimated the performance of *C. angustispirale*: While the normalized root mean square error (see Fig. S3), which quantifies the deviation of the model from the data, of the prediction ranged for most pairs between rather low values of 6 and 21, for this pair it reached a value of 45, indicating a relatively high deviance between modelled and observed data. Under control conditions, *M. elongata* overgrew *C. angustispirale* in the experiment and thus dominated strongly, but under stress, *M. elongata* changed its behaviour and could no longer overgrow *C. angustispirale*. For the sake of simplicity, however, our model does not yet take into account interactions between competition and stress or alternative forms of fungal interactions such as overgrowth.

The priming benefit of *C. angustispirale* predicted by the simulation model was within the range of variation of the observed benefit for all five pairs (Fig. 3B). The model underestimation of growth when competing against *M. elongata* affected both T and PT treatment and thus cancelled out when calculating the relative benefit $b_{si} = \frac{A_{si,PT}}{A_{si,T}}$.

The relative benefit of priming

In four of the six investigated species, the post-lag growth phase was not significantly different from the control growth, and priming did not affect the growth of any of the species. Instead, the duration of the post-stress phase without growth was reduced in five species (see Table 1 and Fig. S2).

When simulating the development of priming benefits over time, a consistent pattern emerged for both isolated and competitive growth (Fig. 3A): The relative benefit increased just after primed *C. angustispirale* restarted growth after the lag phase, and reached a maximum when the non-primed lag phase ended. The subsequent decrease in benefit results from the simultaneous increase of both primed and non-primed colony areas leading to a smaller relative difference between them.

For isolated growth, the immediate benefit was larger than under competition, because an isolated colony could expand without hindrance and benefit strongly from the shortened lag phase, while under competition, a competitor would have already claimed part of the space a species could grow on. The final relative benefit, however, was lowest in isolation, because without competitors there

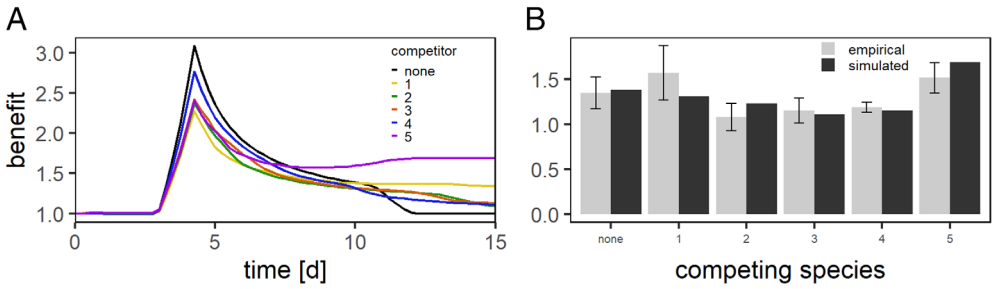

Fig. 3. Priming benefit of *C. angustispirale*.
A. Simulation of the priming benefit of *C. angustispirale* over time in isolation or competition.
B. Comparison of the priming benefit of *C. angustispirale* in isolation or competition with one of five other soil fungi (represented by their competitor number listed in Table 1 and Table S1). Values represent the observed benefit at the last day of measurements and the simulated benefit for the same day.

was no advantage in claiming space earlier, as eventually all available space would be overgrown. The final benefit was largest when *C. angustispirale* faced very competitive, i.e. fast-growing, species (for example, *M. elongata*).

When we systematically varied fungal response traits, for all combinations of traits within the investigated parameter space, priming was beneficial (i.e. relative benefit >1, Fig. 4) 8 days after inoculation, since priming involved no costs. However, under competition with a highly primeable and stress-susceptible competitor, priming was only marginally beneficial, especially when the competing species was fast-growing. Conversely, we observed the highest benefit when *C. angustispirale* faced a stress-resistant and only moderately primeable competitor. Here, the negative effect of fast-growing competitors was reversed, and the relative benefit was highest for a fast-growing competitor (upper right vs. lower left panel of Fig. 4). A very susceptible competitor with high primeability strongly reduced its lag phase under priming. The faster that opponent grows, the more *C. angustispirale* will suffer from its gain in growing time. If, however, the opponent is less primeable, a priming cue will be of no great advantage to that species. In this case, *C. angustispirale* will benefit even more if the competitor is fast-growing.

Fifteen days after inoculation, when the Petri dish was filled and a steady state was reached, priming was not beneficial (i.e. $b_{C.a} < 1$) in case of a fast-growing, highly primeable competitor with intermediate or high stress susceptibility (Fig. S5). During phases of growth, space that is lost to a more primeable competitor can still be compensated by colonizing empty space elsewhere. If space is limited and the competitor is highly primeable, however, it can be more beneficial not to be primed at all.

The effect of priming on competition

Analogous to the investigation of priming benefits, we measured how the colony ratio between *C.*

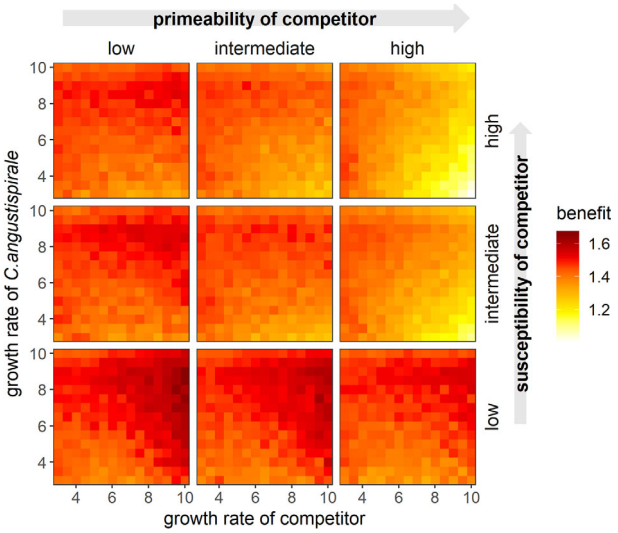

Fig. 4. Priming benefit of *C. angustispirale* in competition with an artificial species. Benefits are shown for different trait combinations 8 days after stress treatment. Levels of susceptibility correspond to different lengths of a stress-induced lag phase: low = 0.5 days, intermediate = 1.5 days, high = 2 days, and levels of primeability correspond to the reduction of this lag phase under priming conditions: low = 25%, intermediate = 50%, high = 100%.

angustispirale and its competitor changed depending on different fungal traits (Fig. 5 and Fig. S6). A positive value indicates that priming favours *C. angustispirale* more than its competitor. Because of its intermediate primeability and high stress susceptibility stemming from a long lag phase in the non-priming treatment, for most investigated trait combinations, *C. angustispirale* benefited stronger than its competitor, as the long stress-induced lag phase of *C. angustispirale* was reduced substantially. If the dual cultures grown experimentally are positioned within Fig. 5 according to the trait values of the competitors, it becomes evident that the effects of priming observed in the laboratory have been moderate. Our model shows that more extreme effects of priming on competition are possible: If competitors show a low

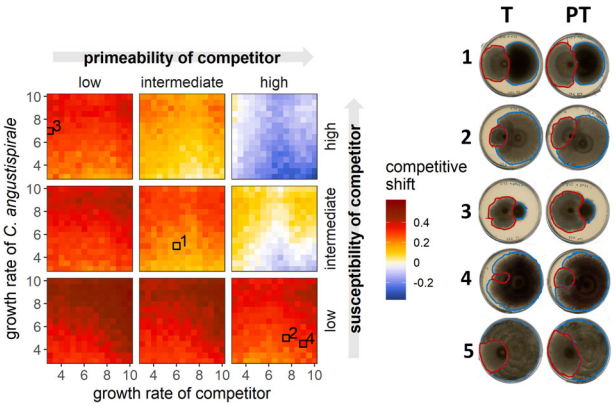

Fig. 5. Competitive shift of *C. angustispirale* in competition with an artificial species. The shifts in competition are shown 8 days after the stress treatment. Red shades indicate a shift in favour of *C. angustispirale*, and blue shades a shift favouring its competitor. Photos show exemplary pairwise cultures grown in the laboratory: Each pair is assigned the respective shift of competition predicted by the simulation model according to the parameter values of the competitor and growth of *C. angustispirale*. The pairs shown are *C. angustispirale* (red) competing against (blue) 1. *F.* sp., 2. An *Amphisphaeriaceae* strain, 3. *P. sapidus*, 4. *F. oxysporum*, 5. *M. elongata*. *M. elongata* is fast-growing and not primeable, and is not represented in the visualized parameter space. Levels of susceptibility correspond to different lengths of a stress-induced lag phase: low = 0.5 days, intermediate = 1.5 days, high = 2 days, and levels of primeability correspond to the reduction of this lag phase under priming conditions: low = 25%, intermediate = 50%, high = 100%.

susceptibility to stress, the benefits of *C. angustispirale* can be much larger than observed in the experiments. If, in contrast, the susceptibility of the competitor is intermediate to high as well as its primeability, the benefits can be reversed leading to a shift towards the competitor.

When comparing Figs 4 and 5, the pattern in both plots seemed to be correlated: indeed, when *C. angustispirale* benefits from priming (i.e. $b_{c.a} > 1$), it will in many cases perform better than its competitor (i.e. $c_{c.a} > 0$), leading to similar patterns in both plots. Nevertheless, in some cases priming conferred a moderate relative benefit to *C. angustispirale*, while the corresponding competitive shift in these cases was variable: facing a primeable but stress susceptible and slow competitor, a competitive shift in favour of the competitor was found (i.e. $c_{c.a} < 0$). If on the other hand the competitor was less primeable or less stress susceptible, a competitive shift in favour of *C. angustispirale* occurred (i.e. $c_{c.a} > 0$), as the competitor benefited less from priming.

Discussion

We have performed laboratory experiments with competitors that cover different growth rates, degrees of primeability and stress susceptibilities. To increase the amount of trait combinations included in our analysis, we successfully developed a cellular automaton model that reproduces the growth of competing fungi in a Petri dish under priming and heat stress conditions. With this model, we varied fungal traits such as stress susceptibility and primeability and assessed how these traits influence the species-specific benefit and competition outcomes.

The priming response of fungi

In our pre-experiments, we showed that while the chosen triggering stress pulse of 45°C affects all six species negatively in their growth (i.e. pushes them away from their optimal growing temperature), it affects them to a different degree. This means that some species will perceive the stress as more severe than others. A stress cue will never affect all members of a community the same, and a priming cue can possibly induce priming in some species but not in others. Nevertheless, we use the term 'community priming' to refer to a setting in which a whole community receives the same mild stress stimulus, which is known to prime at least some of the community members towards a second stronger stress stimulus. As not all species respond equally to both stress stimuli, competition can shift and the community might still change differently than without a priming cue preceding a heat stress. Our approach does not allow for a direct comparison of physiological priming responses between species but instead reflects the way stress priming affects communities in nature.

For all investigated species, a heat-induced no-growth phase was observed in the experimental data, and for four of six species, the post-lag growth phase was not significantly different from the control growth. Priming did not affect the growth of any of the species but instead reduced the duration of the phase without growth. An analytical study by Wesener and Tietjen (2019) using coupled differential equations of microbial growth showed that stress of short duration is best met with an early defence and that a primed stress strategy that further shortens the time until the response is most successful. The current study confirms this pattern, as the fungi were treated with 2-h pulses of heat instead of prolonged periods of warming, and the primed colonies restarted growth earlier than those that had not been primed. Our model captured the dynamics for short stress durations and the effects of priming over several days before interaction types such as overgrowth dominate, while the community response to heat stress of longer duration remains to be investigated. Longer durations of heat stress should be applied as the fungal stress response types will likely differ for different types of heat stress.

Especially for species with a regeneration phase of less than a day (*F. oxysporum* and *F.* sp.) the temporal resolution of measurements after stress should be

increased to enable differentiation between an immediate but slow reversal to control level growth or a lag phase with no growth and 'switch-like' change.

To parameterize the effect of inhibition at a distance, we used control measurements only and applied this parameterization to the other stress treatments. We could show that the inhibiting effect of growth due to a competitor is similar under all stress treatments. However, in some species combinations, heat stress could qualitatively alter the type of interaction between competitors, such as changing overgrowth to deadlock. This is in line with previous findings (Hiscox, Clarkson, *et al*., 2016a) and could be a valuable extension to our simulation model.

Priming costs

In this study, we aimed at accurately imitating the growth dynamics of fungi under priming conditions. We did not implement any costs of priming, as there was no evidence under laboratory conditions that costs of priming are realized as reduced growth. Because priming usually involves the transient production of precursor molecules or transcription factors rather than the accumulation of resistance compounds, priming costs are generally expected to be low (Heil, 2014) and might be hard to quantify. Especially under laboratory conditions, costs of induced resistance can be overseen, e.g. when they manifest as ecological costs (Heil, 2002). We investigated the effect of short-time stress pulses only and we expect the costs of priming to become apparent for a longer duration of stress.

The distribution of resources between growth, resistance and reproduction is central to ecological theory, and any defence strategy must entail some costs (Harvell, 1990; Schulenburg *et al*., 2009; Crowther *et al*., 2014). A priming mechanism without costs would not bear any risks, and even in environments with low stress predictability (leading to organisms reacting to a priming cue, which is not followed by a triggering stress) priming would be of no disadvantage and would be ubiquitous in nature. To our knowledge, there is no study that investigated the costs of priming in microbes. Studies on priming costs in plants differed in their results for different species and priming cues, finding no direct costs of priming (Perazzolli *et al*., 2011), costs realized as growth reduction (Hulten *et al*., 2006), or reduced rhizome production (Yip *et al*., 2019). Priming costs in fungi might thus also not be manifested in reduced growth but rather in reduced spore production or competitive strength. Therefore, we want to stress the need for research on the costs of induced resistance in microbes, which is necessary to fully comprehend the benefits and potential trade-offs of priming.

The benefit of priming

Because we did not implement any priming costs, during the growth phase priming is generally beneficial for *C. angustispirale* in all investigated scenarios. Therefore, we focus rather on the magnitude and not on the presence of this benefit.

Our results show that the relative benefit of priming under competition is highly dependent on fungal traits such as primeability, stress susceptibility and growth, as well as the time point during community build-up. We could show that depending on these factors, priming might not always be more beneficial under competition compared with the isolated benefit. Priming is least beneficial when a species faces a primeable but stress susceptible and competitive (i.e. fast-growing) species. Even when priming itself is relatively beneficial for a given species (i.e. it performs better than without priming), it might still be less competitive under priming conditions depending on the traits of its competitors. Priming can thus be beneficial when taking into account the change of the community structure and the resulting fitness of competing species, making it difficult to infer priming effects in a community from effects measured on species in isolation.

In the future, more traits that influence the effect of priming should be analysed, such as the production of defence compounds. Scaling this production would allow a more dynamic response to the presence of a competitor and could result in different qualitative interactions such as inhibition at a distance or overgrowth - both interaction types are currently not implemented in the model.

Community priming

Chaetomium. *angustispirale* exhibits moderate primeability and shows the longest stress-induced lag phase of the six investigated species. As a result, priming has the potential to strongly shorten its lag phase and thus to be highly beneficial in comparison to its competitors with lower susceptibility or lower primeability. Our model showed that when a steady state is reached and space is limited, priming is generally less advantageous, while during colonization of new territory it can be more beneficial, but also of greater disadvantage when facing primeable competitors. Because the competition for space in fungal communities is effectively competition for gaining access to nutrients (Boddy, 2000), it is of particular importance when colonizing new territory. We showed that a primed stress response that allows an organism to occupy empty space earlier than its competitors leads to the additional advantage of claiming space that would otherwise be colonized by another species. This result can be transferred to higher-order communities, where

the order of species arrival in community assembly affects community structure and function (Fukami, 2015) and priority effects have been shown to be a common influence (Kennedy *et al*., 2009). Because all modelled species compete for the same resources under severe space limitation, these priority effects constitute strong niche preemption (Fukami, 2015). However, our model does not take into account that priority effects can even be of increased importance when species further change environmental conditions or resource availability for later species via niche modification (Fukami, 2015). Environmental factors such as temperature have been shown to influence the assembly of fungal community members (Hiscox *et al*., 2015; Hiscox, Clarkson, *et al*., 2016a; Hiscox, Savoury, *et al*., 2016b). Therefore, heat priming can potentially influence the order of community assembly by letting certain species grow earlier than others.

Priming might not only affect community composition via community assembly but also directly influence community structure: Sensitivity of microbial communities to disturbances is common, as they rarely return to pre-disturbance composition and reach alternative stable states (Shade *et al*. 2012; Schimel, Balser, and Wallenstein 2007; Allison and Martiny 2008). Environments with fluctuating temperatures show an increased species number in fungal communities (Toljander *et al*., 2006), and post-stress communities can transiently consist of species that are generally more resistant to stress (Evans and Wallenstein, 2012; Jurburg *et al*., 2017). Priming, however, can influence community resistance, if less resistant but instead primeable species persist in a community. Stress responses at an individual level, such as priming, might therefore interact with legacy effects arising from pre-disturbance community composition (Meisner *et al*., 2018), resulting in communities with different functions or stress resistance.

Our study advanced the understanding of ecological effects on priming in three ways. First, in our laboratory experiments we found that findings on priming benefits from microbial species in isolation cannot simply be transferred to species competing with other species. Second, the model showed that individual benefits of priming in a community context are highly dependent on the traits of both species and do not necessarily translate into a competitive advantage. And third, although the species chosen in our laboratory experiment showed a wide range of traits, the observed effects on competition were not at all representative of the full spectrum of potential effects as detected in our simulation study. This shows that inferring priming effects on communities from experiments on species in isolation can be highly misleading and that models are a valuable tool to complement laboratory experiments.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Supporting Information.